Leukemia (2007) 21, 622–626
© 2007 Nature Publishing Group All rights reserved 0887-6924/07 $30.00
www.nature.com/leu

CONCISE REVIEW

Why and how to quantify minimal residual disease in acute lymphoblastic leukemia?

T Szczepański[1,2]

[1]Department of Pediatric Hematology and Oncology, Silesian Medical Academy, Zabrze, Poland and [2]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Several studies have demonstrated that monitoring of minimal residual disease (MRD) in childhood and adult acute lymphoblastic leukemia (ALL) significantly correlates with clinical outcome. MRD detection is particularly useful for evaluation of early treatment response and consequently for improved front-line therapy stratification. MRD information is also significant for children undergoing allogeneic hematopoietic stem cell transplantation and those with relapsed ALL. Currently, three highly specific and sensitive methodologies for MRD detection are available, namely multiparameter flow cytometric immunophenotyping, real-time quantitative polymerase chain reaction (RQ-PCR)-based detection of fusion gene transcripts or breakpoints, and RQ-PCR-based detection of clonal immunoglobulin and T-cell receptor gene rearrangements. In this review, characteristics, pitfalls, advantages and disadvantages of each MRD technique are critically discussed. The special emphasis is put on interlaboratory standardization, especially in view of the results obtained within the European collaborative BIOMED-1, BIOMED-2, and Europe Against Cancer projects and recent developments by European Study Group on MRD detection in ALL and EuroFlow Consortium. Standardized MRD techniques form the basis for stratification of patients into the risk groups in new treatment protocols mainly in childhood ALL. Only the results of these studies can answer the question whether MRD-based treatment intervention is associated with improved outcome.
Leukemia (2007) **21,** 622–626. doi:10.1038/sj.leu.2404603; published online 15 February 2007
Keywords: acute lymphoblastic leukemia; minimal residual disease; flow cytometric immunophenotyping; real-time polymerase chain reaction; immunoglobulin and T-cell receptor genes; fusion genes

The rationale for detection of MRD in ALL

The concept of minimal residual disease (MRD) detection in acute lymphoblastic leukemias (ALL) is inherently associated with the progress in treatment of these malignancies.[1] More than 80% of childhood and 35% of adult ALL patients can be cured with modern chemotherapy supplemented with hematopoietic stem cell transplantation (HSCT) in high risk patients (reviewed by Hoelzer *et al.*[2]). Still, a substantial number of ALL patients relapse and the prediction of relapse with conventional prognostic factors such as age, blast count at diagnosis, immunophenotype at diagnosis, presence of chromosome aberrations, response to steroid prophase and classical clinical risk group assignment is far from optimal. Also microarray-based gene expression profiling could not identify gene signatures, typically associated with high risk of relapse. Tracing residual leukemic cells during early phases of treatment provides prognostic information superior to all known classical prognostic factors. Several prospective studies in childhood ALL demonstrated that the most relevant information comes from detection of MRD in bone marrow at the early phases of treatment, particularly at the end of induction treatment (reviewed by Szczepański *et al.*[3] and Cazzaniga and Biondi[4]). Children with undetectable MRD at the end of induction have an excellent prognosis and are good candidates for treatment de-intensification or at least should not be subjected to further treatment intensification, particularly not to HSCT.[5–9] In childhood ALL, a group of ultrafast responders could be identified with successful clearance of MRD in bone marrow even within first 2 weeks of the induction.[10,11] In contrast, children with high MRD levels at the end of induction treatment are in urgent need for treatment intensification or even for novel treatment approaches, particularly when such high MRD levels persist into the consolidation treatment.[5–9] Children with high-risk primary ALL and children with relapsed ALL planned for allogeneic HSCT can also profit from MRD monitoring.[12–17] It is now generally accepted that one of the prerequisites for successful allo-HSCT is maximal reduction of MRD before start of the transplant procedure. Patients with high pretransplant MRD levels are at very high risk for ALL relapse.[12,14,15] Currently, the ongoing studies aim at assessment of possible approaches to lower pretransplant MRD levels and/or attune graft-versus-host reaction according to post-transplant MRD levels by modifying the immunosuppression to improve the outcome after HSCT. Finally, it was recently demonstrated that treatment stratification of standard risk adult ALL patients can be substantially improved when including MRD information.[18]

In conclusion, based on the above-summarized significant results, several prospective treatment protocols, mainly in childhood ALL have been initiated including MRD-based treatment intervention as an essential part. Only the results of these studies can answer the question whether MRD-based treatment intervention is associated with improved outcome.

The methods for quantification of MRD in ALL

Optimal MRD techniques should be characterized by patient specificity (or at least leukemia specificity), satisfactory sensitivity (at least 10^{-4}, i.e., one malignant cell among 10 000 normal cells), applicability for the vast majority of patients under the study, feasibility (easy standardization and rapid collection of results for clinical application) as well as intralaboratory and interlaboratory reproducibility. Another prerequisite for reliable MRD technique is precise quantification of MRD levels. The stringent criteria described above are for the greater part met by three approaches, namely multiparameter flow cytometric immunophenotyping, real-time quantitative polymerase chain

Correspondence: Dr T Szczepański, Department of Pediatric Hematology and Oncology, Silesian Medical Academy, Ul. 3 Maja 13/15, 41-800 Zabrze, Poland.
E-mail: szczep57@poczta.onet.pl
Received 7 January 2007; accepted 10 January 2007; published online 15 February 2007

reaction (RQ-PCR)-based detection of fusion gene transcripts or breakpoints, and RQ-PCR-based detection of clonal immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements (Table 1).[19–21]

Quantification with multiparameter flow cytometric immunophenotyping

Precise quantification is an inherent feature of flow cytometry, which measures single cells. Up till now, immunophenotypic MRD detection in ALL was based on 3–4 color flow cytometry. This methodology relies on tracing the leukemia-specific immunophenotypes as the result of cross-lineage antigen expression, maturational asynchronous expression of antigens, antigen overexpression, absence of antigen expression, ectopic antigen expression, and various combinations of above-mentioned features bringing the ALL blasts into the 'empty spaces' between normal lymphoid differentiation (summarized by Campana[19] and Szczepański *et al*.[20]). Still the detection limit of this technique is not lower than 10^{-3}–10^{-4}, which nevertheless should be sufficient for identifying high-risk ALL patients. Another pitfall of flow cytometry is the modulation of antigen expression occurring during the treatment, which can change the leukemia-specific immunophenotype into a phenotype that resembles that of normal lymphoid precursors.[22,23] Moreover, immunophenotypic changes might occur between diagnosis and relapse.[24] Therefore, following of at least two leukemia-specific immunophenotypes per patient has been recommended to prevent false-negative results. It is striking that the most significant MRD studies in ALL employing flow cytometric immunophenotyping were based on a single expert laboratory.[7,9] This raises the issue of interlaboratory standardization of flow cytometric reagents and procedures, which is essential for international multicenter treatment protocols. With the advent of bench top $\geqslant$6-color flow cytometers, higher sensitivity for MRD monitoring should be achieved, but with the increase of technical complexity the need for interlaboratory standardization becomes even more urgent. In Europe this need for innovation and standardization is supported by the European Commission via the EuroFlow Consortium aiming at standardization of *'Flow cytometry for fast and sensitive diagnosis and follow-up of hematological malignancies'*.[25]

RQ-PCR-based quantification of leukemia-associated fusion genes

Leukemia-associated fusion genes resulting from chromosomal translocations are directly linked to leukemogenesis and therefore represent very good and stable disease-specific markers. After numerous single-center studies, the uniform primers and

Table 1 Characteristics of the techniques currently employed for MRD detection in ALL

	Flow cytometric immunophenotyping	*PCR analysis of chromosome aberrations (mainly detection of fusion gene transcripts)*	*PCR analysis of Ig/TCR genes (junctional region specific approach)*
Sensitivity	10^{-3}–10^{-4}	10^{-4}–10^{-6}	10^{-4}–10^{-5}
Applicability			
Precursor-B-ALL	>95%	40–45%[a]	90–95%
T-ALL	>95%	15–35%[b]	90–95%
Advantages	Applicable for most patients Relatively cheap Rapid: 1–2 days	Relatively easy and cheap Sensitive and leukemia-specific Stable target during disease course Rapid: 2–3 days Suitable for monitoring of uniform patient groups (e.g. *BCR-ABL*+ ALL)	Applicable for virtually all patients, if *IGH*, *IGK*-Kde, *TCRG*, and *TCRD* gene rearrangements are used as targets Sensitive and patient specific Rapid during follow-up: 2–3 days (if junctional region is identified and if RQ-PCR is used)
Disadvantages	Limited sensitivity Need for preferably two aberrant immunophenotypes per patient, because of chance of immunophenotypic shifts Drug-induced modulation of the immunophenotype might influence the levels of antigenic expression	Useful in only a minority of patients Not patient-specific – cross-contamination of PCR products might lead to false-positive results (even at diagnosis) Differences in fusion transcript expression levels between the patients Stability of fusion gene transcripts decreases over time	Time consuming at diagnosis: identification of the junctional regions and sensitivity testing Relatively expensive Need for preferably two PCR targets per patient, because of chance of clonal evolution Two sensitive targets ($\leqslant 10^{-4}$) available in ~80% of patients
Recent developments and standardization in European networks	$\geqslant$6-color cytometry gives promises of increased sensitivity and specificity; currently under development in the European EuroFlow Consortium	Largely standardized thanks to pan-European collaboration within the BIOMED-1 (fusion transcript detection) and EAC project (RQ-PCR) Methods for identification of fusion gene breakpoints at the DNA level provide patient-specific targets	Target identification standardized within the European BIOMED-1 and BIOMED-2 networks RQ-PCR for MRD detection standardized by the European Study Group for MRD detection in ALL (ESG-MRD-ALL)

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; EAC, Europe Against Cancer; Ig, immunoglobulin; *IGH*, immunoglobulin heavy chain gene; *IGK*, immunoglobulin kappa light chain gene; MRD, minimal residual disease; RQ-PCR, real-time quantitative polymerase chain reaction; PCR, polymerase chain reaction; T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor; *TCRD*, T-cell receptor delta gene, TCRG, T-cell receptor gamma gene.
[a]In childhood ALL this particularly concerns t(12;21)(*TEL-AML1*) and in adult ALL particularly t(9;22)(*BCR-ABL*).
[b]This mainly concerns del(1)(p32 p32) with *SIL-TAL1* fusion and t(5;14) with aberrant *HOX11L2* expression, together occurring in 25–35% of childhood T-ALL and in 15-20% of adult ALL (Graux[48]).

protocols for reverse transcription (RT)-PCR for identification of the most frequent fusion transcripts in ALL: t(1;19)(q23;p13) with the *E2A-PBX1* fusion gene, t(4;11)(q21;q23) with the *MLL-AF4* fusion gene, the two main types of t(9;22)(q34;q11) with *BCR-ABL* fusion genes, t(12;21)(p13;q22) with the *TEL-AML1* fusion gene, and the intrachromosomal microdeletion on 1p32 with the *SIL-TAL1* fusion gene, were standardized within the European BIOMED-1 and Europe Against Cancer networks.[26–28] However, these leukemia specific markers can be identified in not more than half of ALL patients, which limits their application as MRD markers for general cohorts of ALL patients. Still, several translocations are significant prognostic markers and identify 'homogenous' ALL subgroups. Both in childhood and adult ALL, t(9;22) with *BCR-ABL* fusion gene is associated with a dismal outcome, although rare cases with a very good sensitivity to chemotherapy could be also identified in this generally drug-resistant subgroup of ALL.[29] With the addition of targeted treatment such as imatinib mesylate to current chemotherapy regimens for *BCR-ABL*$^+$ ALL, *BCR-ABL* transcripts have become the first-choice marker for MRD monitoring by RT-RQ-PCR.[30,31] In contrast, *TEL-AML1* fusion gene occurring in approximately 25% of children with ALL identifies a subgroup with a very good prognosis for which the advantage of MRD monitoring is not yet fully proven.

The protocols for detection and quantification of fusion gene transcripts based on RT-RQ-PCR employing TaqMan technology have been developed thanks to European EAC network.[27] These MRD assays are characterized by reproducibly high sensitivity of 10 plasmid molecules or 10^{-4} RNA cell line dilution for the majority of the targets. The EAC study also selected appropriate reference genes to correct variations in RNA quality and quantity and to calculate the sensitivity of each measurement.[28] These 'control' genes are characterized by highly stable expression in blood and bone marrow of normal and patient samples.

Because of the high sensitivity of PCR techniques, cross-contamination of RT-PCR products between patient samples is a major pitfall in RT-PCR-mediated MRD studies, resulting in up to 20% of false-positive results.[32] Such cross-contamination is difficult to recognize, as fusion gene transcripts, although leukemia-specific, are not patient-specific markers. Also the levels of fusion gene transcripts can vary significantly between patients. This is in contrast to PCR products obtained from genomic breakpoint fusions, which can be identified by use of patient-specific oligonucleotide probes in RQ-PCR assays. Unfortunately, in most translocations occurring in ALL the breakpoints are widely over multiple intronic sequences and their precise identification is rather complex. Nevertheless, the group of Marschalek was able to develop a single standardized approach to identify the vast majority of breakpoint fusions in the many different chromosome translocations involving the *MLL* gene on chromosome 11q23. The DNA breakpoints of *MLL* fusions were shown to be highly specific and sensitive RQ-PCR markers for MRD detection in *MLL*-rearranged infant and adult ALL.[33,34] Such DNA-based approaches for precise breakpoint identification and MRD monitoring should ideally be developed for the other major ALL subgroups with specific chromosome translocations.

RQ-PCR-based quantification of junctional regions of Ig and TCR gene rearrangements

RQ-PCR-based detection and quantification of junctional regions of clonal Ig and TCR gene rearrangements is by far the most widely employed strategy of MRD monitoring in ALL. Although this MRD strategy is the most laborious, expensive and time consuming, it is the most reproducible approach not only within the same laboratory but also between different laboratories. The junctional regions of clonal Ig and TCR gene rearrangements are fingerprint-like sequences for each lymphoid malignancy and can be identified in the vast majority of ALL patients using the standardized primer sets established through the European collaboration within the BIOMED-1 and -2 frameworks.[35–37] The immunobiologic studies identified and characterized oligoclonality and clonal evolution of Ig/TCR gene rearrangements between diagnosis and relapse.[38–42] Therefore, it is widely accepted that preferably at least two Ig/TCR targets should be followed per patient.

Although initial MRD studies employing different semiquantitative approaches, revealed significant results,[5,8,43] only the introduction of RQ-PCR was the major step towards the wider dissemination of this MRD strategy.[21,44] This issue of Leukemia contains an excellent summary of Van der Velden *et al*.[45] on interpretation of RQ-PCR data of MRD monitoring by Ig/TCR gene rearrangements based on the experience of 30 MRD-PCR laboratories forming the European Study Group on MRD detection in ALL (ESG-MRD-ALL). This international initiative has resulted in significantly improved standards, definitions and guidelines for RQ-PCR-based MRD detection via Ig/TCR gene rearrangements. Based on extensive experimentation, this study clearly established definitions of MRD positivity and MRD quantitative range, within which MRD levels can be expressed in a reproducible way. As shown by earlier reports, this quantitative range is usually until 10^{-4} and 10^{-5}, depending on the type of gene rearrangement and the size of junctional region. Another important guideline concerns the discrimination between low MRD positivity outside the quantitative range and false positivity resulting from nonspecific background amplification. Clearly, some Ig/TCR gene rearrangement junctions (particularly of *TCRG*), although unique for a particular patient, are so similar to rearrangements in the normal Ig/TCR repertoire that nonspecific amplification might occur from non-leukemic lymphocytes at low or even moderate levels. Therefore, the sensitivity of this MRD approach cannot easily be further improved. Realizing this, the studies demonstrating frequent MRD positivity in remission samples of children with ALL at the levels of 10^{-7} should be interpreted with extreme caution.

Finally, the report contains additional criteria for interpretation of RQ-PCR data, which take into account whether the clinical protocol aims at therapy intensification or treatment reduction. The conclusion that the ESG-MRD-ALL guidelines for interpretation of RQ-PCR MRD data enable uniform interpretation of MRD data between different MRD laboratories is therefore fully justified.[45]

Choice of MRD technique

The three major MRD techniques provide information expressed as seemingly identical MRD levels. Nevertheless, while flow cytometry relies on protein expression, fusion genes are generally detected at messenger RNA levels, and Ig/TCR junctions are RQ-PCR targets at the DNA level. As summarized in Table 1, the three methodologies also differ in their sensitivity and applicability. Therefore, MRD data obtained with different techniques are hardly interchangeable. Small single-center studies claimed that results of MRD detection by flow cytometry and quantitative PCR of patient-specific Ig/TCR gene rearrangements are largely comparable.[46,47] Indeed in 70–80% of samples with MRD levels $>10^3$ both techniques seem to give comparable results ($\leqslant$3-fold difference). However, in samples

with MRD levels $\leqslant 10^3$, many discrepancies between the two techniques have been found; this hampers the recognition of low-risk patients.[46,47] Consequently, usage of different MRD techniques for different patients within the same treatment protocol should be avoided.

What would be the future of MRD studies in ALL?

The story of MRD is one of the most exciting examples of translational research, where complicated basic research was transferred into high-technology laboratory diagnostics. It is beyond doubt that MRD diagnostics will be included in all ALL treatment protocols, as MRD data provide so far the most optimal reflection of the *in vivo* response to treatment, which gives the clinician a better knowledge and control of the clinical course in individual patients. It is fair to assume that such individualized medicine will finally translate into improved outcome of ALL patients. This is already obvious in case of high-risk ALL patients, particularly those undergoing allogeneic HSCT and still remains to be proved for general cohorts of patients. Introduction of novel, preferably targeted therapy will create additional applications of MRD monitoring. Still ongoing international collaborative efforts are necessary to ensure that all diagnostic MRD laboratories speak the same 'MRD language'.

Acknowledgements

The author is supported by scientific Grants number 2P054 095 30 and 3PO5E 094 25 from the Polish Ministry of Science and Higher Education.

References

1 Szczepański T, Orfao A, van der Velden VHJ, San Miguel JF, van Dongen JJM. Minimal residual disease in leukaemia patients. *Lancet Oncol* 2001; **2**: 409–417.
2 Hoelzer D, Gokbuget N, Ottmann O, Pui CH, Relling MV, Appelbaum FR *et al.* Acute lymphoblastic leukemia. *Hematology (Am Soc Hematol Educ Program)* 2002, 162–192.
3 Szczepański T, Flohr T, van der Velden VHJ, Bartram CR, van Dongen JJM. Molecular monitoring of residual disease using antigen receptor genes in childhood acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol* 2002; **15**: 37–57.
4 Cazzaniga G, Biondi A. Molecular monitoring of childhood acute lymphoblastic leukemia using antigen receptor gene rearrangements and quantitative polymerase chain reaction technology. *Haematologica* 2005; **90**: 382–390.
5 Van Dongen JJM, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L *et al.* Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet* 1998; **352**: 1731–1738.
6 Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J *et al.* Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. *N Engl J Med* 1998; **339**: 591–598.
7 Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC *et al.* Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet* 1998; **351**: 550–554.
8 Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N *et al.* Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. *Blood* 2002; **99**: 1253–1258.
9 Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N *et al.* Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood* 2002; **99**: 1952–1958.
10 Panzer-Grumayer ER, Schneider M, Panzer S, Fasching K, Gadner H. Rapid molecular response during early induction chemotherapy predicts a good outcome in childhood acute lymphoblastic leukemia. *Blood* 2000; **95**: 790–794.
11 Coustan-Smith E, Sancho J, Behm FG, Hancock ML, Razzouk BI, Ribeiro RC *et al.* Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. *Blood* 2002; **100**: 52–58.
12 Knechtli CJ, Goulden NJ, Hancock JP, Grandage VL, Harris EL, Garland RJ *et al.* Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. *Blood* 1998; **92**: 4072–4079.
13 Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Proba J *et al.* Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. *Lancet* 2001; **358**: 1239–1241.
14 Van der Velden VHJ, Joosten SA, Willemse MJ, van Wering ER, Lankester A, van Dongen JJM *et al.* Real-time quantitative PCR for detection of minimal residual disease before allogeneic stem cell transplantation predicts outcome in children with acute lymphoblastic leukemia. *Leukemia* 2001; **15**: 1485–1487.
15 Bader P, Hancock J, Kreyenberg H, Goulden NJ, Niethammer D, Oakhill A *et al.* Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post transplant outcome in children with ALL. *Leukemia* 2002; **16**: 1668–1672.
16 Krejci O, van der Velden VHJ, Bader P, Kreyenberg H, Goulden N, Hancock J *et al.* Level of minimal residual disease prior to haematopoietic stem cell transplantation predicts prognosis in paediatric patients with acute lymphoblastic leukaemia: a report of the Pre-BMT MRD Study Group. *Bone Marrow Transplant* 2003; **32**: 849–851.
17 Coustan-Smith E, Gajjar A, Hijiya N, Razzouk BI, Ribeiro RC, Rivera GK *et al.* Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia after first relapse. *Leukemia* 2004; **18**: 499–504.
18 Bruggemann M, Raff T, Flohr T, Gokbuget N, Nakao M, Droese J *et al.* Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* 2006; **107**: 1116–1123.
19 Campana D. Determination of minimal residual disease in leukaemia patients. *Br J Haematol* 2003; **121**: 823–838.
20 Szczepański T, van der Velden VH, van Dongen JJM. Flow-cytometric immunophenotyping of normal and malignant lymphocytes. *Clin Chem Lab Med* 2006; **44**: 775–796.
21 Van der Velden VHJ, Hochhaus A, Cazzaniga G, Szczepański T, Gabert J, Van Dongen JJM. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia* 2003; **17**: 1013–1034.
22 Gaipa G, Basso G, Maglia O, Leoni V, Faini A, Cazzaniga G *et al.* Drug-induced immunophenotypic modulation in childhood ALL: implications for minimal residual disease detection. *Leukemia* 2005; **19**: 49–56.
23 Van der Sluijs-Gelling AJ, van der Velden VHJ, Roeffen ETJM, Veerman AJP, van Wering ER. Immunophenotypic modulation in childhood precursor-B-ALL can be mimicked *in vitro* and is related to the induction of cell death. *Leukemia* 2005; **19**: 1845–1847.
24 Van Wering ER, Beishuizen A, Roeffen ET, van der Linden-Schrever BE, Verhoeven MA, Hahlen K *et al.* Immunophenotypic changes between diagnosis and relapse in childhood acute lymphoblastic leukemia. *Leukemia* 1995; **9**: 1523–1533.
25 Orfao A, López A, Flores J, Almeida J, Vidriales B, Perez J *et al.* Diagnosis of hematological malignancies: new applications for flow cytometry. *Hematology (EHA Educ Program)* 2006; **2**: 6–13.
26 Van Dongen JJM, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G *et al.* Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; **13**: 1901–1928.
27 Gabert J, Beillard E, Van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N *et al.* Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in

leukemia – a Europe Against Cancer program. *Leukemia* 2003; **17**: 2318–2357.
28 Beillard E, Pallisgaard N, van der Velden VH, Bi W, Dee R, van der Schoot E *et al*. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe against cancer program. *Leukemia* 2003; **17**: 2474–2486.
29 Arico M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A *et al*. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. *N Engl J Med* 2000; **342**: 998–1006.
30 Scheuring UJ, Pfeifer H, Wassmann B, Bruck P, Gehrke B, Petershofen EK *et al*. Serial minimal residual disease (MRD) analysis as a predictor of response duration in Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL) during imatinib treatment. *Leukemia* 2003; **17**: 1700–1706.
31 Wassmann B, Pfeifer H, Stadler M, Bornhauser M, Bug G, Scheuring UJ *et al*. Early molecular response to posttransplantation imatinib determines outcome in MRD+ Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood* 2005; **106**: 458–463.
32 Gleissner B, Rieder H, Thiel E, Fonatsch C, Janssen LA, Heinze B *et al*. Prospective BCR-ABL analysis by polymerase chain reaction (RT-PCR) in adult acute B-lineage lymphoblastic leukemia: reliability of RT-nested-PCR and comparison to cytogenetic data. *Leukemia* 2001; **15**: 1834–1840.
33 Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S *et al*. The MLL recombinome of acute leukemias. *Leukemia* 2006; **20**: 777–784.
34 Burmeister T, Marschalek R, Schneider B, Meyer C, Gokbuget N, Schwartz S *et al*. Monitoring minimal residual disease by quantification of genomic chromosomal breakpoint sequences in acute leukemias with MLL aberrations. *Leukemia* 2006; **20**: 451–457.
35 Pongers-Willemse MJ, Seriu T, Stolz F, d'Aniello E, Gameiro P, Pisa P *et al*. Primers and protocols for standardized MRD detection in ALL using immunoglobulin and T cell receptor gene rearrangements and *TAL1* deletions as PCR targets. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; **13**: 110–118.
36 Van Dongen JJM, Langerak AW, Bruggemann M, Evans PAS, Hummel M, Lavender FL *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 2003; **17**: 2257–2317.
37 Szczepański T, Van der Velden VHJ, Hoogeveen PG, de Bie M, Jacobs DC, van Wering ER *et al*. Vdelta2-Jalpha rearrangements are frequent in precursor-B-acute lymphoblastic leukemia but rare in normal lymphoid cells. *Blood* 2004; **103**: 3798–3804.
38 Szczepański T, Willemse MJ, van Wering ER, Weerden JF, Kamps WA, van Dongen JJM. Precursor-B-ALL with DH-JH gene rearrangements have an immature immunogenotype with a high frequency of oligoclonality and hyperdiploidy of chromosome 14. *Leukemia* 2001; **15**: 1415–1423.
39 De Haas V, Verhagen OJ, von dem Borne AE, Kroes W, van den Berg H, van der Schoot CE. Quantification of minimal residual disease in children with oligoclonal B-precursor acute lymphoblastic leukemia indicates that the clones that grow out during relapse already have the slowest rate of reduction during induction therapy. *Leukemia* 2001; **15**: 134–140.
40 Szczepański T, Willemse MJ, Brinkhof B, van Wering ER, van der Burg M, van Dongen JJM. Comparative analysis of Ig and TCR gene rearrangements at diagnosis and at relapse of childhood precursor-B-ALL provides improved strategies for selection of stable PCR targets for monitoring of minimal residual disease. *Blood* 2002; **99**: 2315–2323.
41 Germano G, del Giudice L, Palatron S, Giarin E, Cazzaniga G, Biondi A *et al*. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. *Leukemia* 2003; **17**: 1573–1582.
42 Szczepański T, Van der Velden VHJ, Raff T, Jacobs DC, van Wering ER, Bruggemann M *et al*. Comparative analysis of T-cell receptor gene rearrangements at diagnosis and relapse of T-cell acute lymphoblastic leukemia (T-ALL) shows high stability of clonal markers for monitoring of minimal residual disease and reveals the occurrence of second T-ALL. *Leukemia* 2003; **17**: 2149–2156.
43 Brisco MJ, Condon J, Hughes E, Neoh SH, Sykes PJ, Seshadri R *et al*. Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction. *Lancet* 1994; **343**: 196–200.
44 Pongers-Willemse MJ, Verhagen OJHM, Tibbe GJM, Wijkhuijs JM, De Haas V, Roovers E *et al*. Real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia using junctional regions specific TaqMan probes. *Leukemia* 1998; **12**: 2006–2014.
45 Van der Velden VHJ, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer R *et al*. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia* 2007; **21**, (in press).
46 Malec M, Bjorklund E, Soderhall S, Mazur J, Sjogren AM, Pisa P *et al*. Flow cytometry and allele-specific oligonucleotide PCR are equally effective in detection of minimal residual disease in ALL. *Leukemia* 2001; **15**: 716–727.
47 Neale GAM, CoustanSmith E, Pan Q, Chen X, Gruhn B, Stow P *et al*. Tandem application of flow cytometry and polymerase chain reaction for comprehensive detection of minimal residual disease in childhood acute lymphoblastic leukemia. *Leukemia* 1999; **13**: 1221–1226.
48 Graux C, Cools J, Michaux L, Vandenberghe P, Hagemeijer A. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. *Leukemia* 2006; **20**: 1496–1510.